MACS

Miltenyi Biotec

Magnetic isolation and fast screening

Exosome research solutions

JOIN THE FLOW REVOLUTION

Isolating exosomes the easy way

No centrifugation required

Isolation of extracellular vesicles from cell culture supernatant or body fluids

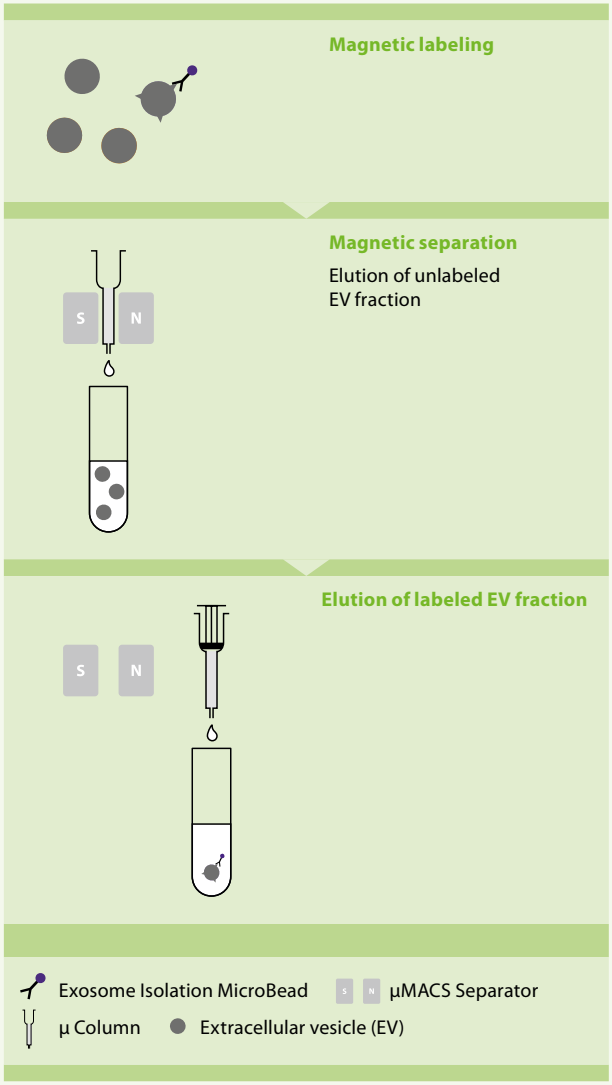

Figure 1: Principle of magnetic isolation of EVs using an Exosome Isolation Kit.

Exosomes – small vesicles, big impact

Exosomes are extracellular vesicles (EVs) of endocytic origin, released by numerous cell types including T cells, B cells, dendritic cells, platelets, neurons, and epithelial cells. They play a key role in a variety of cellular functions, including coagulation and intercellular signaling. There is growing interest in applying exosomes in clinical settings as they can potentially be used as biomarkers, for example.

MACS® Technology – the proven technique for magnetic separation

EVs can be easily isolated by MACS® Technology – the tried and tested cell isolation method cited in tens of thousands of publications. This technique owes its longstanding success to the combination of three components: i) nano-sized superparamagnetic MACS MicroBeads coupled to antibodies specifically detecting certain epitopes on the surface of cells or EVs, ii) MACS Separators, and iii) MACS Columns generating a strong magnetic field.

Miltenyi Biotec offers dedicated kits for isolating EVs based on the tetraspanin proteins CD9, CD63, and CD81, which are known to be present on the surface of exosomes. Exosome Starting Kits contain all materials required for convenient exosome isolation, including separator and columns.

Isolation of EVs in three easy steps

First, EVs contained in cell culture supernatant or body fluids are magnetically labeled with Exosome Isolation MicroBeads CD9, CD63, or CD81. The labeled EVs are then loaded onto a µ Column, which is placed in the magnetic field of a µMACS™ Separator. Magnetically labeled EVs are retained within the column, while unlabeled vesicles and cell components run through. After removing the column from the magnetic field, the intact EVs can be eluted (fig. 1).

- Fast (< 2h) and easy isolation of exosomes without ultracentrifugation
- Targeted isolation based on CD9, CD63, or CD81, or all three makers combined
- EV isolation from cell culture supernatant or body fluids like plasma, urine, or ascites

MACSPlex Exosome Kit

Featured application

Fast and reliable EV isolation and protein profiling

EVs isolated with Exosome Isolation Kits based on single tetraspanins, i. e., CD9, CD63, or CD81, can be easily analyzed for their protein profiles using the MACSPlex Exosome Kit (fig. 4). For most of the MACSPlex Exosome Capture Bead types, EVs isolated based on CD63 gave the strongest signals, followed by CD81 and CD9. EVs are traditionally prepared by ultracentrifugation. However, this method is time consuming and can lead to inconclusive results in protein profiling experiments (fig. 4): EVs isolated by ultracentrifugation showed only weak fluorescence signals compared to EVs isolated by MACS® Technology, when analyzed with the MACSPlex Exosome Kit.

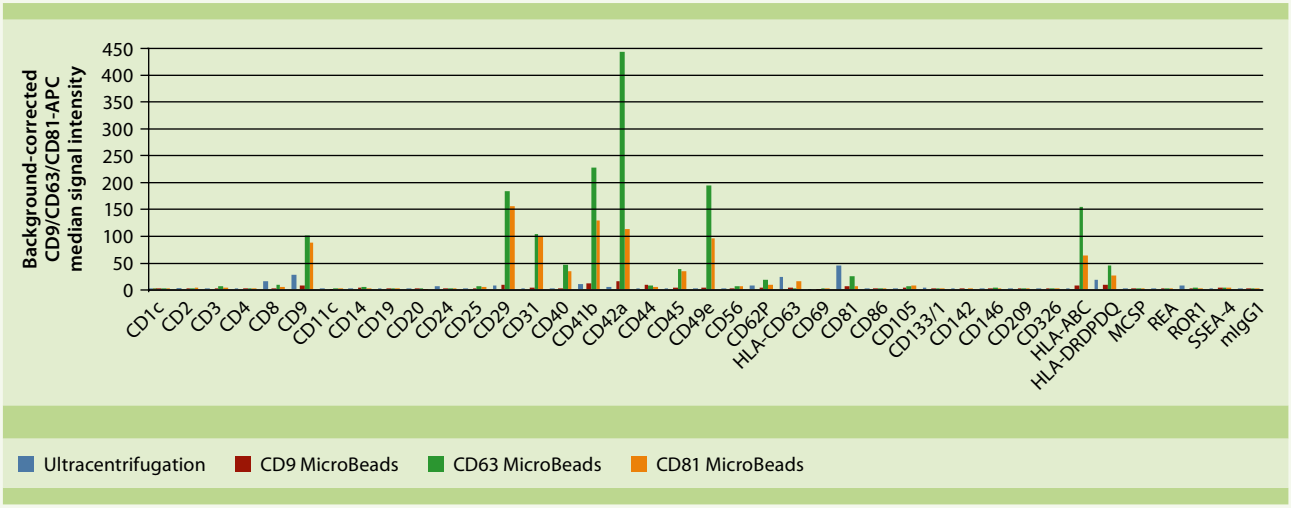

Figure 4: Surface marker profiles of EVs isolated from plasma by ultracentrifugation or immunomagnetic isolation using the Exosome Isolation Kits CD9, CD63, or CD81. EVs from 2 mL of plasma were isolated using one of the kits or by ultracentrifugation. Amounts were adjusted to a plasma volume of 2 mL, and exosomes were analyzed using the MACSPlex Exosome Kit. Data indicate median APC signal intensities of isolated EVs incubated with the 39 MACSPlex Exosome Capture Beads and stained with a cocktail of CD9-, CD63-, and CD81-APC antibodies. REA and mIgG1 indicate isotype control beads.

Product	Order no.
Exosome Isolation Kit CD9, human	130-110-913
Exosome Isolation Kit CD63, human	130-110-918
Exosome Isolation Kit CD81, human	130-110-914
Exosome Isolation Kit Pan, human	130-110-912
Exosome Starting Kit CD9, human	130-111-573
Exosome Starting Kit CD63, human	130-111-576
Exosome Starting Kit CD81, human	130-111-575
Exosome Starting Kit Pan, human	130-111-572

Product	Order no.
Exosome Isolation Kit CD9, mouse	130-117-042
Exosome Isolation Kit CD63, mouse	130-117-041
Exosome Isolation Kit CD81, mouse	130-117-040
Exosome Isolation Kit Pan, mouse	130-117-039
Exosome Starting Kit CD9, mouse	130-118-917
Exosome Starting Kit CD63, mouse	130-118-915
Exosome Starting Kit CD81, mouse	130-118-916
Exosome Starting Kit Pan, mouse	130-118-918
MACSPlex Exosome Kit, human	130-108-813

Table 1: Products for exosome research.

MACSPlex Exosome Kit

Featured application

Antibodies for EV analysis		
Anti-HLA-ABC	CD19	CD62P
Anti-HLA-DR, DP, DQ	CD20	CD63
Anti-MCSP	CD24	CD69
Anti-ROR1	CD25	CD81
Anti-SSEA-4	CD29	CD86
CD1c	CD31	CD105
CD2	CD40	CD133/1
CD3	CD41b	CD142
CD4	CD42a	CD146
CD8	CD44	CD209
CD9	CD45	CD326
CD11c	CD49e	Mouse IgG1 Control
CD14	CD56	REA Control

Table 2: Overview of surface marker and control antibodies used for EV analysis by the MACSplex Exosome Kit.

EVs from isolated primary blood cell types

Protein profiling of EVs released by specific blood cell types or subsets thereof requires a reliable and effective method for the isolation of the individual cell populations.

MACS MicroBeads are the ideal solution enabling the enrichment of distinct cell types, even directly from whole blood. The isolated cells are immediately ready for cell culture, and EVs that are released into the culture medium can then directly be analyzed by the MACSplex Exosome Kit.

Figure 5 shows a protein profile of EVs that were released from isolated immune cells. Many markers that are expressed on the surface of a particular cell type are also present on EVs released by these cells. Therefore, marker profiles of EVs can be used to pinpoint their origin. Table 2 provides an overview of the antibodies used for EV analysis.

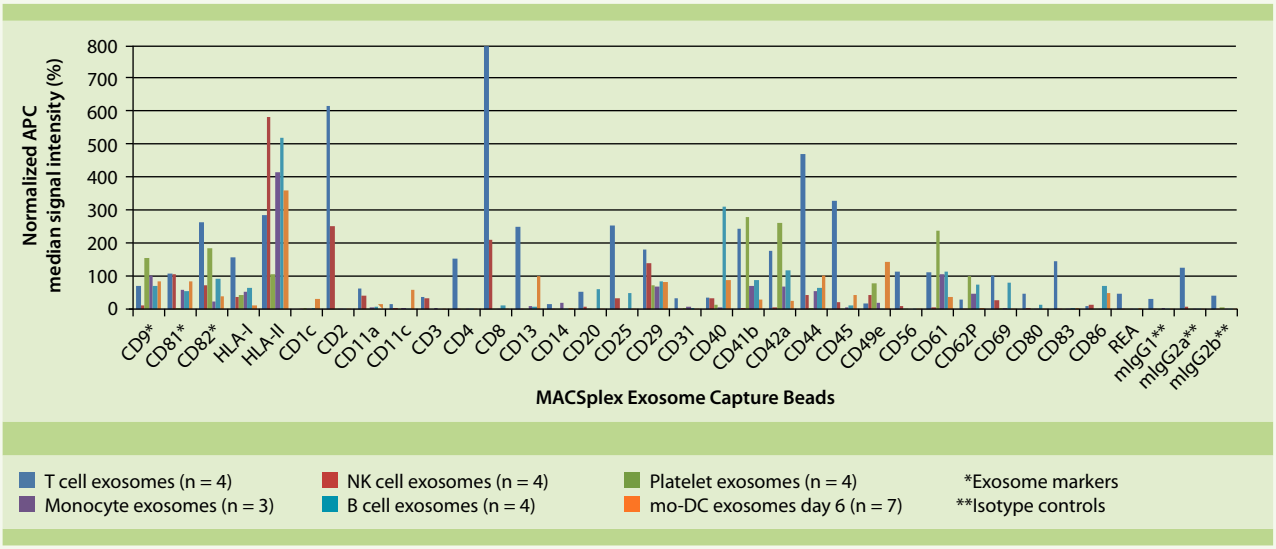

Figure 5: Characterization of EVs released from isolated blood cell types. Individual cell types were isolated from blood of healthy donors, using cell type–specific MACS MicroBeads. After short-term culture of the isolated cells, EVs released into the culture supernatant were analyzed using MACSplex Exosome Capture Beads most of which are contained in the MACSplex Exosome Kit. Monocyte-derived dendritic cells (Mo-DCs) were differentiated from CD14+ monocytes. To compensate for different EV yields, signal intensities were normalized to the mean of the exosome markers CD9 and CD81, which was defined as 100%.

New horizons for exosome analysis

Fast screening by flow cytometry

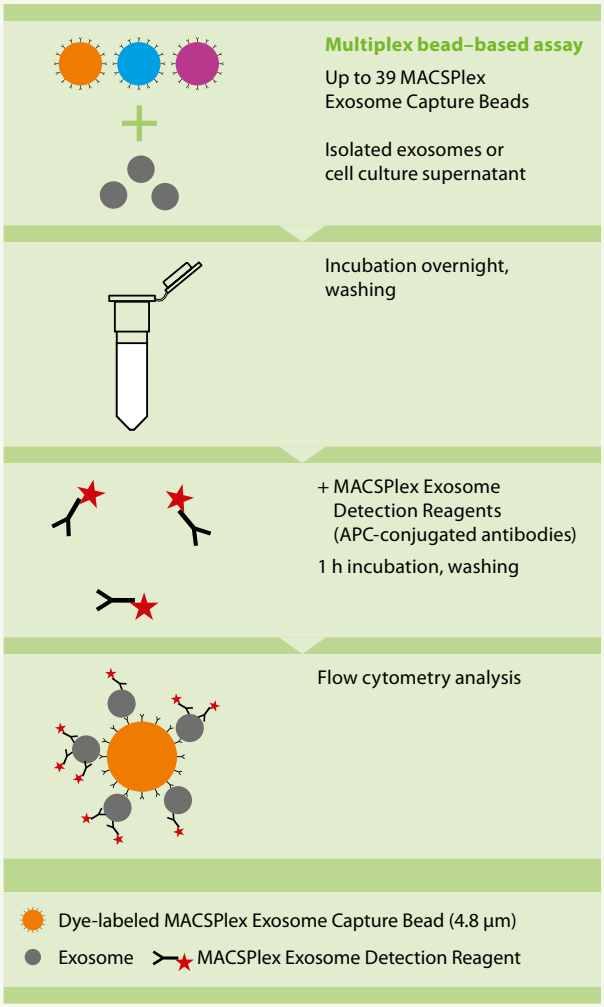

Figure 2: Principle of the MACSPlex Exosome Kit. Isolated exosomes are incubated overnight with 39 differently labeled MACSPlex Exosome Capture Beads each coupled to a different antibody. Exosomes bound to the beads are detected with MACSPlex Exosome Detection Reagents by flow cytometry.

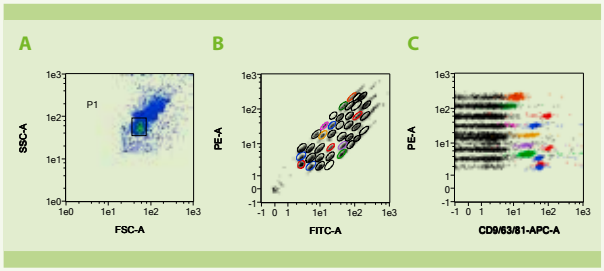

Figure 3: Exosome analysis based on the MACSPlex Exosome Kit. The example shows **(A)** gating according to bead size, **(B)** discrimination of differently labeled bead populations, and **(C)** measurement of signal intensities of the single bead populations.

MACSPlex Exosome Kit – the solution for comprehensive analysis

Due to their small size, it has been difficult thus far to analyze EVs by standard flow cytometry, which hampered scientific advancement in this field. To facilitate comprehensive EV analysis, Miltenyi Biotec developed the MACSPlex Exosome Kit. This novel tool enables an easy and fast screening of potential EV surface proteins.

- Unique multiplex bead platform for protein profiling of EVs by flow cytometry
- Saves precious sample material by screening 37 surface markers simultaneously
- Analysis of EVs from cell culture supernatant or body fluids

Principle of the MACSPlex Exosome Kit

The MACSPlex Exosome Kit allows for the simultaneous detection of 37 surface epitopes that are known to be present on different EVs. The kit is based on a cocktail of various fluorescently labeled bead populations, the MACSPlex Exosome Capture Beads, which can be distinguished by flow cytometry.

Each of these MACSPlex Exosome Capture Bead populations is coupled to a specific antibody binding to a respective exosomal surface epitope. Exosomes bound to the MACSPlex Capture Beads are stained with MACSPlex Exosome Detection Reagents, i.e., a cocktail of APC-conjugated antibodies against the tetraspanins CD9, CD63, and CD81, for example. This leads to the formation of complexes, each consisting of i) MACSPlex Exosome Capture Bead, ii) exosome, and iii) APC-conjugated antibodies (fig. 2). These complexes can then be analyzed based on the fluorescence characteristics of both the MACSPlex Exosome Capture Beads and the APC-conjugated antibodies (fig. 3). Two isotype control beads are included in the kit to check for non-specific binding. Analysis is made easy by an express mode on MACSQuant® Flow Cytometers. This express mode simplifies setup, execution, and analysis of an experiment so that even users with minimal experience in flow cytometry can perform complex exosome analyses.

▸ miltenyibiotec.com/exosome

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